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EXPRESSION OF THE GREEN FLUORESCENT PROTEIN CARRIED BY *AUTOGRAPHA CALIFORNICA* MULTIPLE NUCLEOPOLYHEDROVIRUS IN INSECT CELL LINES

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SUMMARY

A recombinant AcMNPV containing the green fluorescent protein (*gfp*) gene under the polyhedrin promoter (*polh*) was used to investigate the expression of the *gfp* gene as well as the production of recombinant extracellular virus in 14 continuous insect cell lines, including *Heliothis virescens* (BCIRL-HV-AM1), *Helicoverpa zea* (BCIRL-HZ-AM1), *Anticarsia gemmatalis* (BCIRL-AG-AM1), *Trichoplusia ni* (TN-CL1), *Spodoptera frugiperda* (IPLB-SF21), *Spodoptera exigua* (BCIRL/AMCY-Se-E1 and BCIRL/AMCY-Se-E5), *Bombyx mori* (BMN), Sf9 (a clone of IPLB-SF21), and five cell line clones of BCIRL-HV-AM1. The susceptibility of the cell lines to the recombinant virus (AcMNPV.GFP) was ascertained by calculating the mean percentage number of green light–emitting cells as well as by $TCID_{50}$ titration of extracellular virus with fluorescence as a sign of infection. Of the 14 cell lines tested, all were permissive with varying degrees to AcMNPV.GFP, except BCIRL-HV-AMCL2 and BCIRL-HZ-AM1, both grown in serum-containing medium, and BMN, grown in serum-free medium, which were nonpermissive to the virus. Except for BCIRL/AMCY-Se-E1, IPLB-SF21, and four of the five BCIRL-HV-AM1 clones, all the other cell lines (BCIRL-HV-AM1, BCIRL-AG-AM1, TN-CL1, Se-E5, and Sf9) expressed detectable levels of GFP by 48 h postinoculation. The BCIRL/AMCY-Se-E1 and IPLB-SF21 cells, grown in serum-free medium (Ex-Cell 401), expressed detectable levels of GFP at 72 h postinoculation. By contrast, in BCIRL/AMCY-Se-E1 in serum-containing medium (Ex-Cell 401 + 10% FBS [fetal bovine serum]), GFP was detected at 48 h postinoculation. Furthermore, TN-CL1 cells produced the largest mean percentage number of fluorescent (76.6%) cells in both serum-containing and serum-free medium (64.8%) at 120 h postinoculation. All the BCIRL-HV-AM1 clones showed no GFP expression until 96 h postinoculation, and only then about 1% of the cell population fluoresced. The mean extracelluar virus (ECV) production at 120 h postinoculation was highest in BCIRL/AMCY-Se-E5 cells grown in Ex-Cell 401 + 10% FBS (37.8×10^6 $TCID_{50}$/ml) followed by BCIRL-HV-AM1 in TC199-MK (33.4×10^6 $TCID_{50}$/ml). Only the BCIRL-HV-AMCL3 clone produced any substantial level of ECV at 120 h postinoculation (16.9×10^6 $TCID_{50}$/ml). However, there was no significant correlation between ECV production and the mean percentage number of fluorescent cells. This study provides further information on the susceptibility of 14 insect cell lines to a recombinant AcMNPV containing the green fluorescent protein gene. This information might avail researchers with information to facilitate decisions as to what other cell lines are available for in vitro studies of the *gfp* gene.

Key words: recombinant baculovirus; AcMNPV; insect cell lines; green fluorescent protein.

INTRODUCTION

The isolation and characterization of the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* (Shimomura et al., 1962; Morin and Hastings, 1971; Ward et al., 1980), the cloning and sequencing of the wild-type *gfp* gene (Prasher et al., 1992), and its incorporation into the *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) (Eriksson et al., 1996; Laukkanen et al., 1996; Reilaender et al., 1996; Fanxiu et al., 1997) has provided researchers with a unique fluorescent expression system that requires no exogenous substrates or cofactors that can be used in a variety of in vivo and in vitro studies. Expression of the *gfp* gene by a recombinant AcMNPV has been studied in only a few insect cell lines, such as the fall armyworm *Spodoptera frugiperda* (Eriksson et al., 1996; Laukkanen et al., 1996; Oker at al., 1996; Reilaender et al., 1996; Wilson et al., 1997; Wu et al., 1997) and the cabbage looper *Trichoplusia ni* (Laukkanen et al., 1996). The need for information on the host range of a baculovirus is an important consideration if the development, production, and use of the baculovirus as a biological control agent is to be successful. With this in mind, the expression of the *gfp* gene by a recombinant baculovirus can facilitate determining the host range of the virus by more easily distinguishing whether successful infection and replication of a cell line has occurred, and, in particular, in a semipermissive cell line, in which it might be difficult to observe a cytopathology effect or occlusion body (OB) production (in the case of the *gfp*

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TABLE 1

INSECT CELL LINES USED IN THIS STUDY

Insect species	Cell line designation	Passage number for each replicate	Explant	Growth medium[a]	Reference
Spodoptera exigua	BCIRL/AMCY-Se-E5	75	Embryonic	Ex-Cell 401 + 10% FBS[b]	Goodman, unpublished
Spodoptera exigua	BCIRL/AMCY-Se-E5	67	Embryonic	Ex-Cell 401 (SFM)[c]	Goodman, unpublished
Spodoptera exigua	BCIRL/AMCY-Se-E1	72	Embryonic	Ex-Cell 401 + 10% FBS	Goodman, unpublished
Spodoptera exigua	BCIRL/AMCY-Se-E1	55, 58, 53	Embryonic	Ex-Cell 401 (SFM)	Goodman, unpublished
Spodoptera frugiperda	Sf9	100	Pupal ovaries	Ex-Cell 401 (SFM)	Summers and Smith, 1987
Spodoptera frugiperda	IPLB-SF-21	346	Pupal ovaries	Ex-Cell 401 (SFM)	Vaughn et al., 1977
Bombyx mori	BMN	59	Unknown	Ex-Cell 401 (SFM)	Maeda et al., 1990
Trichoplusia ni	TN-CL1	519, 530, 530	Ovarian tissue	Ex-Cell 401 (SFM)	Hink, 1970
Trichoplusia ni	TN-CL1	518, 532, 532	Ovarian tissue	Ex-Cell 401 + 10% FBS	Hink, 1970
Helicoverpa zea	BCIRL-HZ-AM1	152	Ovarian tissue	TC-199-MK[d]	McIntosh and Ignoffo, 1983
Heliothis virescens	BCIRL-HV-AM1	299, 301, 301	Pupal ovaries	TC-199-MK[d]	McIntosh et al., 1981
Heliothis virescens	BCIRL-HV-AM1	554	Pupal ovaries	Ex-Cell 401 + 10% FBS	McIntosh et al., 1981
Anticarsia gemmatalis	BCIRL-AG-AM1	122, 118, 122	Ovarian	TC-199-MK[d]	McIntosh and Ignoffo, 1989
Heliothis virescens	BCIRL-HV-AMCL1	9	Pupal ovaries	Ex-Cell 401 + 10% FBS	McIntosh, unpublished
Heliothis virescens	BCIRL-HV-AMCL2	24	Pupal ovaries	Ex-Cell 401 + 10% FBS	McIntosh, unpublished
Heliothis virescens	BCIRL-HV-AMCL3	11	Pupal ovaries	Ex-Cell 401 + 10% FBS	McIntosh, unpublished
Heliothis virescens	BCIRL-HV-AMCL5	14	Pupal ovaries	Ex-Cell 401 + 10% FBS	McIntosh, unpublished
Heliothis virescens	BCIRL-HV-AMCL7	28	Pupal ovaries	Ex-Cell 401 + 10% FBS	McIntosh, unpublished

[a] 50 μg of streptomycin per milliliter and 50 units of penicillin per milliliter were added to each growth medium.
[b] Ex-Cell 401 is a serum-free insect cell culture medium (JRH Biosciences, Lenexa, KS). FBS = fetal bovine serum.
[c] SFM = serum-free medium.
[d] TC-199-MK is an insect cell culture medium (Sigma Chemical Co., St Louis, MO) and was supplemented with 20% FBS.

gene under the control of a promoter other than the polyhedrin promoter). Testing of the recombinant baculovirus containing the *gfp* gene in other cell lines might also provide further information on the capabilities of particular cell lines to express a foreign protein that would enable workers to select an optimum cell line tailored to their particular research needs. In this study, we used a recombinant AcMNPV containing the *gfp* gene under the polyhedrin promoter (*polh*) to investigate the expression of the *gfp* gene as well as the production of recombinant extracellular virus in 14 continuous insect cell lines, including six cell line clones.

Materials and Methods

Insect cell lines and media. A description of various cell lines and media used in this study is found in Table 1. The cell lines were maintained as monolayer cultures in Falcon® T-25-cm² plastic flasks. All the cell lines were previously identified by DAF-PCR (DNA Amplification Fingerprinting)-PCR (McIntosh et al., 1996) with the exception of the following five additional clones of BCIRL-HV-AM1, which were identified by DAF in this study: BCIRL-HV-AMCL1, BCIRL-HV-AMCL2, BCIRL-HV-AMCL3, BCIRL-HV-AMCL5, and BCIRL-HV-AMCL7.

Establishment of Spodoptera exigua *cell lines. Spodoptera exigua* eggs were obtained from Ken Johnson (Ecogen, Inc., Langhorne, PA). Insects were maintained in our laboratory using established procedures (Berger, 1963; Ignoffo, 1965; Ignoffo and Boening, 1970). Cultures were initiated from eggs (a minimum of 24 h old). Eggs were sterilized using 70% ethanol (containing 1% Triton X-100), followed by 0.53% sodium hypochlorite (Clorox Co., Oakland, CA), then rinsed twice in Hanks' Balanced Salt Solution (HBSS; containing 200 units/ml penicillin, 0.2 mg/ml streptomycin, 0.5 μg/ml amphotericin B, 25 m*M* MOPS, pH 6.8) (5 min/wash). The sterilized eggs were homogenized in HBSS using a plastic micropestle in a 1.5-ml Eppendorf tube. Homogenates were centrifuged to pellet the cells (500 × *g*, 10 min), brought up to 5 ml with ExCell 401™ (JRH Biosciences, Lenexa, KS; containing 10% FBS, penicillin, and streptomycin, as previously described) and placed into disposable T_{25} flasks (Stiles et al., 1992). To discourage melanization, 0.7 mg/ml glutathione was added to the medium (Goodman et al., 1982), followed by frequent medium changes within the first wk. Primary cultures were initially fed every 7 d (½ medium replacement), then once or twice a wk as they progressed (total medium replacement).

Virus. Extracellular virus (ECV) of the recombinant AcMNPV (AcMNPV.GFP) containing the *gfp* gene under the control of the *polh* promoter was used in the infectivity studies. Wilson et al. (1997) has provided a detailed description of the construction of the recombinant AcMNPV.GFP used in this study.

Inoculation of cell lines with AcMNPV.GFP extracellular virus. Selected lines were seeded at 1×10^5 cells/ml in 5 ml of medium in each of three T-25-cm² flasks and allowed to attach for 2 h at room temperature. The old medium was removed and replaced with 1 ml of fresh medium, and cells were then inoculated with AcMNPV.GFP ECV at a multiplicity of infection of 0.03. Cells were rocked for 2 h on a Bellco rocker platform (Bellco Technology, Vineland, NJ) at a setting of 2 at room temperature to allow for virus attachment. Medium was then removed, the cells were washed twice with 5 ml of HBSS to remove any residual inoculum, 5 ml of fresh medium were added, and cells were incubated at 28° C.

Determination of percentage infected cells and extracellular virus production. The percentage of cells infected with AcMNPV.GFP was determined by counting the number of green light–emitting cells within a randomly selected region of the microscopic field for each of three replicate flasks at 24-h intervals from 24 to 120 h postinoculation using an IM 35 Epifluorescence Microscope (Carl Zeiss, Thornwood, NY) and barrier filters exciting at 485 nm with emission at 510 nm. The titer of the supernatant collected after 120 h postinoculation that contained ECV for each cell line was determined with TN-CL1 as the indicator cell line in Ex-Cell 401 + 10% FBS, according to a previously described method (McIntosh et al., 1983). Dilution end-point assays, based on the number of positive wells that contained green light–emitting cells, were performed on the indicator cell line in duplicate and results recorded after 7 d incubation at 28° C. A computer program written in BASIC employing the Spearman–Karber method was used to calculate the $TCID_{50}$ values (Lynn, 1992), and the SigmaStat® program was used to statistically analyze the data.

Results

Cytopathology. All the cell lines previously reported to be permissive for the wild-type AcMNPV, such as BCIRL-HV-AM1 (McIntosh et al., 1985; McIntosh and Ignoffo, 1989), BCIRL-AG-

TABLE 2

MEAN PERCENTAGE INFECTION BASED ON THE NUMBER OF GREEN LIGHT–EMITTING CELLS INFECTED BY THE RECOMBINANT AcMNPV.GFP[a]

Cell line[b]	Medium	Time (h) postinoculation				
		24	48	72	96	120
BCIRL/AMCY-Se-E1	Ex-Cell 401 (SFM)	0	0	NR	0.67 (0.37–1.2)	1.7 (0.12–4.6)
BCIRL/AMCY-Se-E1	Ex-Cell 401 + 10% FBS	0	0.3 (0–0.8)	8.5 (5.9–10.1)	48.1 (42.3–58.8)	57.5 (52.9–65.8)
BCIRL/AMCY-Se-E5	Ex-Cell 401 + 10% FBS	0	NR	NR	2.6 (1.4–3.6)	24.0 (20.3–31)
BCIRL-AG-AM1	TC-199-MK	0	3.5 (2.7–4.6)	17.3 (5.9–33.9)	39.5 (22.9–69.1)	43.7 (26.8–69.2)
BCIRL-HV-AM1	TC-199-MK	0	NR	6.2 (1.0–11.6)	9.4 (1.2–17.6)[c]	20.8 (10.6–26.8)
BCIRL-HV-AM1	Ex-Cell 401 + 10% FBS	0	TFIC	TFIC	35.5 (28.0–39.5)	32.8 (25.5–42.8)
TN-CL1	Ex-Cell 401 + 10% FBS	0	2.3 (0.9–5.8)[c]	41.4 (21.3–58.1)	73.4 (57.8–93.1)	76.6 (66.2–88.8)
TN-CL1	Ex-Cell 401 (SFM)	0	0.2 (0–0.47)[c]	2.0 (0.24–3.5)	29.5 (14.7–55.1)	64.8 (49.2–76.4)
Sf9	Ex-Cell 401 (SFM)	0	NR	0.06 ((0–0.2)	0.13 (0–0.4)	4.5 (1.6–9.6)
IPLB-SF21	Ex-Cell 401 (SFM)	0	0	TFIC	TFIC	TFIC

[a] Means calculated from three replicates; cells in three randomly selected fields of view were counted for each replicate.

[b] BCIRL-HZ-AM1, BMN, and BCIRL/AMCY-Se-E5 cells in Ex-Cell 401 (SFM) showed no cytopathology or GFP production. NR = green light–emitting cells were observed but were not counted, as the field of view containing these cells was not randomly selected. A range of values of percentage infection for each cell line is shown in parentheses. An MOI of 0.03 was used to initiate infection.

[c] Only two replicates were counted. TFIC = too few green light–emitting cells to count and calculate a percentage infection value.

AM1 (McIntosh and Ignoffo, 1989), IPLB-SF-21 (McIntosh et al., 1985) and its clone Sf9 (Summers and Smith, 1987), and TN-CL1 (McIntosh et al., 1985; McIntosh and Ignoffo, 1989), based on OB formation and $TCID_{50}$ titration, showed the typical cytopathic effect of rounding cells and a granular appearance characteristic of a baculovirus infection by 24 h postinoculation with AcMNPV.GFP. Two newly established *Spodoptera exigua* cell lines, BCIRL/AMCY-Se-E1 and BCIRL/AMCY-Se-E5, also showed cytopathetic effects similar to those previously reported in studies employing other *Spodoptera exigua* cell lines (Gelernter and Federici, 1986; Smits and Vlak, 1988). Some of the cells—namely, BCIRL/AMCY-Se-E1 in Ex-Cell 401 + 10% FBS, BCIRL/AMCY-Se-E5 in Ex-Cell 401 + 10% FBS, BCIRL-AG-AM1, BCIRL-HV-AM1, TN-CL1 in both serum-free and serum-containing media, and Sf9 in Ex-Cell 401 (SFM)—showed green light–emitting cells at 48 h postinoculation. In contrast, it took an additional 24 h before IPLB-Sf21 cells were observed to express detectable levels of GFP. Variation in the intensity of emission of green light was also observed among individual cells within a given cell line. As expected, controls showed no background level of green fluorescence.

Percentage infection based on the number green light–emitting cells. The mean percentage infection of various cells by the recombinant AcMNPV.GFP is shown in Table 2. Again, all the cell lines that were found to be permissive for the wild-type AcMNPV in previous studies were also susceptible to the recombinant AcMNPV.GFP. The TN-CL1 cells grown in Ex-Cell 401 + 10% FBS showed the highest percentage infection of any of the cell lines tested at 96 h (73.4%) and 120 h (76.6%) postinoculation. It took a further 24 h for the same cell line grown in Ex-Cell401(SFM) to reach a comparable percentage number of green light–emitting cells at 120 h postinoculation. The BCIRL/AMCY-Se-E1 cell line was the only other one to show a high proportion of green light–emitting cells (57.5%) at 120 h postinoculation. Of the five BCIRL-HV-AM1 clones tested, BCIRL-HV-AMCL2 was nonpermissive to the recombinant virus, whereas the remaining four clones did not express the GFP protein until 96 h postinoculation, when only less than 1% of the cells were fluorescent.

Extracellular virus production based on $TCID_{50}$ assays. Extracellular virus production, measured at 120 h postinoculation, was highest in BCIRL/AMCY-Se-E5 (37.81×10^6 $TCID_{50}$/ml) and BCIRL-HV-AM1 (33.4×10^6 $TCID_{50}$/ml) cell lines followed by BCIRL/AMCY-Se-E1 (17.3×10^6 $TCID_{50}$/ml) (Table 3). There was no statistically significant difference ($P < 0.05$) among these cell lines, BCIRL-HV-AM1 grown in Ex-Cell 401 + 10% FBS (8.86×10^6 $TCID_{50}$/ml), and BCIRL-AG-AM1 grown in TC199-MK (15.1×10^6 $TCID_{50}$/ml). The TN-CL1 cells produced the lowest level of virus (4.42×10^6 $TCID_{50}$/ml) of all the cell lines grown in serum-containing medium, whereas Sf9 cells produced the highest level of the virus (4.81×10^6 $TCID_{50}$/ml) of all the cell lines grown in serum-free medium. Moreover, IPLB-SF21 had the significantly lowest ECV production of all the cell lines tested ($P < 0.05$). Production of ECV among four of the five BCIRL-HV-AM1 clones was not significantly different ($P = 0.066$), whereas BCIRL-HV-AMCL2 was apparently nonpermissive to this virus, as no green light–emitting cells were observed, and no ECV was produced (Table 4). There was also no significant relationship between the level of ECV produced and percentage number of green light–emitting cells at 120 h postinoculation based on the Spearman rank order correlation test ($P > 0.05$).

Discussion

The results of this study demonstrate that the recombinant AcMNPV containing the *gfp* gene under the polyhedrin promoter (*polh*) is capable of producing a differential infectious response in seven different continuous insect cell lines, as measured by percentage green light–emitting cells and ECV production. Furthermore, it would appear from this study that some of the insect cell lines frequently used by researchers, such as IPLB-SF21, its clone Sf9, and TN-CL1, may not be as suitable for the production of this recombinant virus, especially if this virus is to be employed in studies as an intracellular fluorescent marker or to amass a stock of recombinant baculovirus ECV for other research purposes. The BCIRL-HV-AM1 cell line grown in TC199-MK and the BCIRL/

TABLE 3

EXTRACELLULAR VIRUS PRODUCTION (ECV)[a] IN CELL LINES BASED ON THE END-POINT DILUTION ASSAY OF GREEN LIGHT–EMITTING CELLS INFECTED BY THE RECOMBINANT AcMNPV.GFP

Cell line	Medium	$TCID_{50}/ml \times 10^6$			Mean $TCID_{50}/ml \times 10^6 \pm$ SEM
		Replicate 1	Replicate 2	Replicate 3	
BCIRL/AMCY-Se-E1	Ex-Cell 401 (SFM)	6.19	0.83	0.15	2.39 ± 1.9 cd
BCIRL/AMCY-Se-E5	Ex-Cell 401 (SFM)	0.91	0.83	0.29	0.68 ± 0.2 d
BCIRL/AMCY-Se-E1	Ex-Cell 401 + 10% FBS	14.68	17.78	19.57	17.3 ± 1.4 a
BCIRL/AMCY-Se-E5	Ex-Cell 401 + 10% FBS	34.81	51.09	21.54	37.81 ± 10.4 a
BCIRL-AG-AM1	TC-199-MK	34.81	6.19	4.22	15.1 ± 9.9 ab
BCIRL-HV-AM1	TC-199-MK	56.23	26.10	17.78	33.4 ± 11.7 a
BCIRL-HV-AM1	Ex-Cell 401 + 10% FBS	9.08	7.50	10.0	8.86 ± 0.7 ab
TN-CL1	Ex-Cell 401 + 10% FBS	8.25	2.87	2.15	4.42 ± 1.9 bcd
TN-CL1	Ex-Cell 401 (SFM)	5.11	2.61	2.61	3.44 ± 0.8 bcd
Sf9	Ex-Cell 401 (SFM)	5.11	4.21	5.11	4.81 ± 0.3 bcd
IPLB-SF21	Ex-Cell 401 (SFM)	0.09	0.35	0.10	0.18 ± 0.1 d

[a] The Kruskal–Wallis ANOVA on Ranks test and Student–Newman–Keuls multiple comparison test were used to compare $TCID_{50}$ values between the cell lines. Cell lines with the same letter are not significantly different at the 0.05 level of significance. Cell cultures were harvested at 120 h postinoculation for ECV titer determinations.

TABLE 4

EXTRACELLULAR VIRUS PRODUCTION (ECV)[a] IN THREE CLONES OF BCIRL-HV-AM1 BASED ON THE END-POINT DILUTION ASSAY OF GREEN LIGHT–EMITTING CELLS INFECTED BY THE RECOMBINANT AcMNPV.GFP

Cell line[b]	Medium	$TCID_{50}/ml \times 10^6$			Mean $TCID_{50}/ml \times 10^6 \pm$ SEM
		Replicate 1	Replicate 2	Replicate 3	
BCIRL-HV-AM-CL1	Ex-Cell 401 + 10% FBS	0.06	0.02	0.09	0.056 ± 0.02
BCIRL-HV-AM-CL3	Ex-Cell 401 + 10% FBS	0.01	0.013	0.03	0.02 ± 0.01
BCIRL-HV-AM-CL5	Ex-Cell 401 + 10% FBS	14.68	26.10	10.0	16.93 ± 8.28
BCIRL-HV-AM-CL7	Ex-Cell 401 + 10% FBS	0.29	0.47	0.002	0.25 ± 0.14

[a] The Kruskal–Wallis ANOVA on Ranks test was used to compare the $TCID_{50}$ values between the different clones. There was no significant difference in ECV production among the clones ($P = 0.066$).

[b] BCIRL-HV-AM-CL2 was nonpermissive to AcMNPV.GFP.

TABLE 5

COMPARISON OF EXTRACELLULAR VIRUS (ECV) PRODUCTION OF WILD-TYPE ACMNPV IN INSECT CELL LINES BASED ON THE MEAN $TCID_{50}$ VALUES (± SEM) REPORTED IN VARIOUS PUBLICATIONS

Insect species	Cell line designation	$TCID_{50}$	Reference
Trichoplusia ni	TN-368	$10^{8.1 \pm 0.37}$	Danyluk and Maruniak, 1987
Trichoplusia ni	IAL-TND	$10^{7.5 \pm 0.14}$	Danyluk and Maruniak, 1987
Spodoptera frugiperda	IPLB-SF-21AE	$10^{7.6 \pm 0.19}$	Danyluk and Maruniak, 1987
Spodoptera frugiperda	IAL-SFD	$10^{6.5 \pm 0.46}$	Danyluk and Maruniak, 1987
Anticarsia gemmatalis	UFL-AG-286	$10^{5.77 \pm 0.58}$	Sieburth and Maruniak, 1988
Spodoptera frugiperda	IPLB-SF-21AE	$10^{7.3 \pm 0.26}$	Sieburth and Maruniak, 1988
Trichoplusia ni	TN-CL1	$10^{7.2 \pm 0.13}$	Sieburth and Maruniak, 1988
Heliothis virescens	BCIRL-HV-AM1	$10^{7.5 \pm 0.10}$	McIntosh and Ignoffo, 1989
Anticarsia gemmatalis	BCIRL-AG-AM	$10^{6.5 \pm 0.12}$	McIntosh and Ignoffo, 1989
Plutella xylostella	BCIRL-PX2-HNV3	$10^{6.4 \pm 0.01}$	McIntosh and Ignoffo, 1989
Spodoptera frugiperda	IPLB-SF21	$10^{6.6 \pm 0.09}$	McIntosh and Ignoffo, 1989
Trichoplusia ni	TN-CL1	$10^{8.8\,a}$	Ignoffo et al., 1998

[a] This is a quantity of ECV determined for a single batch of in vitro produced wild-type AcMNPV consisting of 10 ml of final whole culture.

AMCY-Se-E5 cell line grown in Ex-Cell 401 + 10% FBS were both shown in this study to produce substantially greater amounts of ECV than TN-CL1 grown in serum-free and serum-containing media as well as IPLB-SF21 and Sf9. Of course, previous investigations have already shown that cell culture medium is also an important abiotic factor to consider in order to obtain optimal cell culture growth as well as both recombinant virus production and expression of foreign proteins (Godwin et al., 1990; Hink, 1991; Chen et al., 1993; McIntosh and Grasela, 1993; McIntosh et al., 1995). In this study, the level of AcMNPV.GFP extracellular virus produced by a low

multiplicity of infection (MOI), at least in BCIRL/AMCY-Se-E1 and BCIRL-HV-AM1 cells, is higher in serum-containing media (TC199-MK; Ex-Cell 401 + 10% FBS) than in serum-free medium (Ex-Cell 401 [SFM]). Furthermore, the data presented here show that unless in vitro infection studies are conducted using some sort of quantitative measure such as $TCID_{50}$ titration, it would be difficult to predict exactly how well a recombinant virus containing the *gfp* gene will respond to different cell lines grown in a particular cell culture medium. For example, an investigation by Joon Cha et al. (1997), employing AcMNPV with the *gfp* gene as a fluorescent protein marker, showed that under their laboratory conditions, Sf9 cells grown in Hink's TNM-FH Insect Medium produced an average infectious titer of 5×10^7 pfu/ml, or about 7.25×10^7 $TCID_{50}$/ml after 7 d incubation. This is about 15 times greater than the titer we obtained in our study (7.25×10^7 $TCID_{50}$/ml versus 4.81×10^6 $TCID_{50}$/ml) using the same cell line and may be a reflection of their use of a different cell culture medium and possibly their use of a larger MOI. The range of $TCID_{50}$ values obtained for the recombinant virus used in this study were comparable to those values previously reported for wild-type AcMNPV (Table 5). The green fluorescent protein marker also facilitated distinguishing positive from negative wells of 96-well plates in the $TCID_{50}$ titration of ECV, as also noted by Joon Cha et al. (1997).

Our results show that the infectivity of the AcMNPV.GFP in a parental cell line such as BCIRL-HV-AM1 does not necessarily indicate that its clones have a similar susceptibility to the recombinant virus. All the BCIRL-HV-AM1 clones produced low ECV titers, except BCIRL-HV-AMCL3, which produced about twice the amount of ECV than did the parental cell line. Various other studies have also shown, though not with recombinant viruses, that there can be substantial differences between parental cell lines and their clones in their susceptibility to wild-type baculovirus (Corsaro and Fraser, 1987; Lenz et al., 1991).

There was no significant predictive relationship between the percentage number of green light–emitting cells and ECV production at 120 h postinoculation. Thus, at this late time period in the virus infection cycle, one is as likely to observe a given infected host cell line that produces a high $TCID_{50}$ titer that can exhibit either a low or a high percentage number of green light–emitting cells. On the other hand, it is also possible to have a low $TCID_{50}$ titer exhibiting either a low or a high percentage number of green light–emitting cells.

The results of the present study demonstrate that some of the insect cell lines—namely, BCIRL-HV-AM1, BCIRL-AG-AM1, and BCIRL/AMCY-Se-E5—are capable of producing high ECV titers of AcMNPV.GFP and might be a more suitable choice than some of the more commonly used cell lines, such as SF21, Sf9, and TN-CL1, especially if the objective of the research is to produce a GFP recombinant virus as a fusion product with another foreign protein under a different promoter.

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